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Climate change may exacerbate the risk of invasiveness of non-native aquatic plants: the case of the Pannonian and Mediterranean regions of Croatia

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Abstract

Non-native aquatic plants are amongst the major threats to freshwater biodiversity and climate change is expected to facilitate their further spread and invasiveness. To date, in Croatia, no complete list of non-native extant and horizon aquatic plants has been compiled nor has a risk screening been performed. To address this knowledge gap, 10 extant and 14 horizon aquatic plant species were screened for their risk of invasiveness in the Pannonian and Mediterranean regions of Croatia under current and predicted (future) climate conditions. Overall, 90% and 60% of the extant species were classified as high risk for the Pannonian and Mediterranean regions, respectively, under both climate scenarios. Of the horizon species, 42% were classified as high risk under current conditions and, under climate change, this proportion increased to 78%. The 'top invasive' species (i.e. scored as very high risk) under both climate conditions and for both regions were extant *Elodea nuttallii* and horizon *Lemna aequinoctialis*. The horizon *Hygrophila polysperma* was very high risk for the Mediterranean Region under current climate conditions and for both regions under projected climate conditions. *Azolla filiculoides*, *Elodea canadensis*, *Egeria densa* and *Utricularia gibba* were also classified as high risk under current climate conditions and, after accounting for climate change, they became of very high risk in both regions. Further, *Gymnocoronis spilanthoides* and *Lemna minuta* were found to pose a very high risk under climate change only for the Pannonian Region. It is anticipated that the outcomes of this study will contribute to knowledge of the invasiveness of aquatic plants in different climatic regions and enable prioritisation measures for their control/eradication.

Keywords
Adriatic Sea Basin, AS-ISK, Black Sea Basin, freshwater, risk screening

Introduction

Invasive non-native species pose globally one of the most serious environmental threats due to their adverse impacts on the environment (Blackburn et al. 2014; Essl et al. 2019; Rendeková et al. 2019) and the resulting multiple socio-economic implications (Lovell et al. 2006; Bacher et al. 2018). Freshwater ecosystems are especially vulnerable to the introduction of invasive non-native species, which occurs via several pathways and vectors linked to a large variety of human activities (Banha and Anastácio 2015; Coughlan et al. 2017; Rodriguez-Merino et al. 2018). In the case of non-native aquatic plants, the main introduction vectors include ship fouling, hitchhiking, fish stocking, floods and other natural events, host and vector organisms, the ornamental trade and aquarium waste releases (e.g. Leung et al. 2006; Pollux et al. 2006; Dehnen-Schmutz and Touza 2008; Hussner et al. 2010; Reynolds et al. 2015). Once established, non-native aquatic plants may alter habitat condition and ecosystem function (Rodriguez-Merino et al. 2018), as well as food-web structure (Villamagna and Murphy 2010), increase the risk of flooding events by impeding river flow (Thouvenot et al. 2013), induce oxygen depletion (Caraco et al. 2006), disrupt ecosystem properties such as soil cover, nutrient cycling, fire regimes and hydrology (Weidlich et al. 2020) and change macroinvertebrate and fish species richness and abundance (Strayer 2010). In addition, non-native aquatic plants mainly reproduce and spread by vegetative propagation (Eckert et al. 2016; Crane et al. 2019), which facilitates their transportation by water currents to new water bodies (Hussner et al. 2017).

In the last 100 years, the number and abundance of non-native aquatic plants has considerably increased worldwide (Hussner et al. 2010). This increase has been mainly caused by enhanced trading, higher water turbidity by eutrophication/re-oligotrophication and by climatic factors mostly related to temperature increase (Hussner et al. 2010; Rodríguez-Merino et al. 2018). Yet, the increased threat posed by new introductions of non-native aquatic plants may still be prevented, or at least mitigated, using horizon scanning as an early-warning tool, which helps to identify (potentially) invasive non-native species that are not yet established within some geographical area often of high conservation value (Copp et al. 2007; Amanatidou et al. 2012; Roy et al. 2015).

In Croatia, the first comprehensive list of aquatic plants, including many rare and threatened species, together with information on their historical and recent distribution, was recently produced from herbarium museum sheets and includes 76 species, of which three are non-native, namely *Azolla filiculoides*, *Egeria densa* and *Elodea canadensis* (Zeko et al. 2020). *Elodea canadensis* had already been introduced to Croatia in the 19th century, specifically 60 years before its first official record in 1954, whereas its congener *Elodea nuttallii* was first recorded in 2006 in the drainage channels of the Kopački Rit Nature Park in the Pannonian Region (Black Sea Basin) of Croatia (Kočić et al.

2014; Nikolić 2022). *Elodea nuttallii* is included in the Invasive Alien Species List of European Union concern (EU 2014) together with *Myriophyllum heterophyllum*, which was discovered in 2000 on Krk Island in the Mediterranean Region (Adriatic Sea Basin) of Croatia and with *Ludwigia peploides*, which was found in 2018 in the Pannonian Region (Jasprica et al. 2017; Buzjak and Sedlar 2018). Finally, *Najas graminea* also was discovered recently in the Mediterranean Region (Glasnović et al. 2015).

In addition to the above, extensive monitoring research on non-native species, including aquatic plants, has been conducted in recent years in Croatia (Kutleša et al. 2021). Based on this monitoring programme, 20 aquatic and semi-aquatic non-native plant species have been documented in the Pannonian Region, whereas the Mediterranean Region remains unexplored. As a result, to date no complete list of non-native aquatic plants has been compiled for Croatia nor a risk screening for their invasiveness in the country has been performed. To address this knowledge gap, the aims of this study were: (i) to identify extant non-native aquatic plant species in Croatia and perform a horizon scanning to find which species might enter Croatia in the future from neighbouring countries; and (ii) to evaluate the risk of invasiveness of the identified extant and horizon species under both current and predicted (future) climate conditions in the Pannonian and Mediterranean regions of Croatia, which belong to two different climate zones. It is anticipated that the outcomes of this study will support the prioritisation of future management measures for introduced non-native aquatic plants in Croatia and will help in the identification of the highest-risk species likely to invade Croatian aquatic ecosystems in the near future with the aim to establish rapid control/eradication measures.

Materials and methods

Study area

Croatia is biogeographically divided into the lowland Pannonian Region, the Mediterranean Region (along the Adriatic coast and in its immediate hinterland) and the highland Alpine area (in the elevated Lika and Gorski Kotar). Hydrologically, the Pannonian Region (a.k.a. Pannonian Plain or Hungarian Lowland) includes the Danube River Basin, which is dominated by the large rivers Danube, Drava and Sava, and the karst Mediterranean region, which includes the Adriatic Sea Basin with its immediate and confined basins (Potočki et al. 2021). The karst rivers of the immediate river basins of the Mediterranean Region have direct confluence into the Adriatic Sea, whereas confined basins represent the highland region with karst rivers (mostly intermittent) without direct confluence to the Adriatic Sea (Fig. 1A). The rivers of the Mediterranean Region are short and isolated and often flow through deep canyons, where they create waterfalls and lakes (cf. lentic expansions). These rivers have a seasonal hydrological regime with abundance of water in autumn and spring, but with some of them drying out completely in summer (Bonacci and Andrić 2008; Bonacci and Roje-Bonacci 2012; Bonacci et al. 2014).

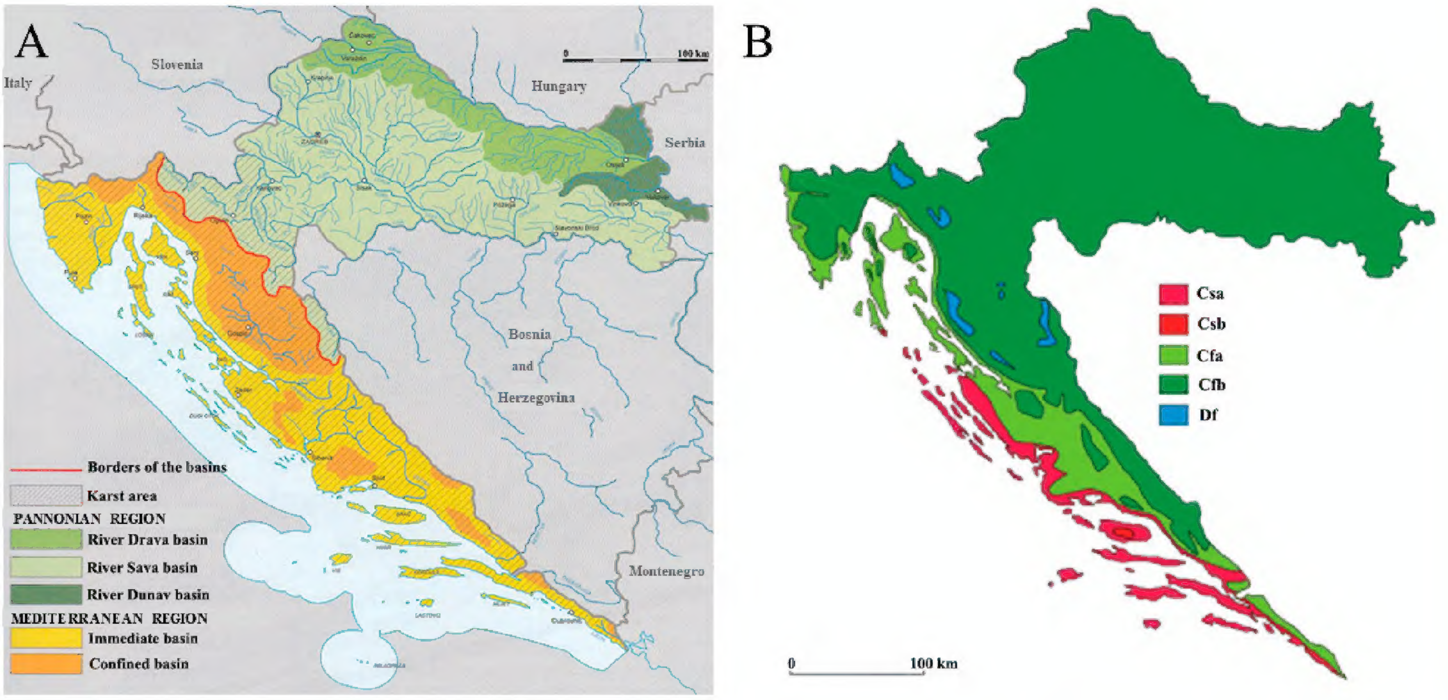

Figure 1. A map of the Pannonian and Mediterranean regions of Croatia representing the two risk assessment areas for the screening of non-native aquatic plants (see Table I) **B** geographical distribution of the climate types in Croatia (according to the Köppen-Geiger climate classification system): *Csa* = warm-temperate with dry and hot summer; *Csb* = warm-temperate with dry and warm summer; *Cfa* = warm-temperate, fully humid, hot summer; *Cfb* = warm-temperate, fully humid, warm summer; *Df* = boreal humid.

Croatia has mostly a temperate rainy climate with average monthly temperature higher than –3 °C and lower than 18 °C in the coldest month. In the Pannonian Region, the warmest month of the year has an average temperature lower than 22 °C, whereas in the Mediterranean Region, it is higher than 22 °C and more than four months in a year have a monthly average temperature higher than 10 °C (Zaninović et al. 2008). According to the Köppen–Geiger climate classification system (Peel et al. 2007), in the Pannonian Region, the *Cfb* climate type (warm-temperate, fully humid, warm summer) prevails, whereas at higher altitudes, this is true of the *Df* climate type (boreal humid). In the Mediterranean Region, the *Cfa* (warm-temperate, fully humid, hot summer) and *Cfb* climate types prevail in the North, whereas the islands and coastal areas of the middle and southern Adriatic Sea are characterised by the *Csa* (warm-temperate with dry and hot summer) and *Csb* (warm-temperate with dry and warm summer) climate types. Finally, the inland area and nearby shores are mostly *Cfa*, changing further from the coast into *Cfb* and *Df* at the highest altitudes (Fig. 1B).

Risk screening

In total, 24 non-native aquatic plant species were selected for risk screening of their potential invasiveness in the Pannonian and Mediterranean regions of Croatia – hereafter, also referred to as the 'risk assessment areas'. The scientific names, authority and more frequently used common names for the screened species are listed in Table 1 and plant nomenclature follows The Plant List (http://www.theplantlist.org/), World Flora Online

Table 1. Extant and horizon non-native aquatic plant species screened for their potential risk of invasiveness in the Pannonian and Mediterranean regions of Croatia. For each species, the Region of establishment (M = Mediterranean; P = Pannonian) is provided together with the *a priori* categorisation outcome into Non-invasive and Invasive (after Vilizzi et al. 2022). GISD = Global Invasive Species Database (www.iucngisd.org); CABI = Centre for Agriculture and Bioscience International Invasive Species Compendium (www.cabi.org/ISC); IESNA = the Invasive and Exotic Species of North America list (www.invasive.org); Gscholar = Google Scholar literature search (whenever applicable). N = no impact/threat; Y = impact/threat; '–' = absent; n.e. = not evaluated (but present in database); n.a. = not applicable.

Species name	*A priori* categorisation						
	Common name	Region	GISD	CABI	IESNA	GScholar	Outcome
Extant							
Azolla cristata Kaulf.	–	P	–	Y	–	n.a.	Invasive
Azolla filiculoides Lam.	Pacific mosquitofern	P	–	Y	–	n.a.	Invasive
Egeria densa Planch.	Brazilian waterweed	M	Y	Y	Y	n.a.	Invasive
Elodea canadensis Michx.	Canadian waterweed	P	Y	Y	–	n.a.	Invasive
Elodea nuttallii (Planch.) H.St John	western waterweed	P	–	Y	–	n.a.	Invasive
Ludwigia peploides (Kunth) P.H.Raven	floating primrose-willow	P	–	Y	–	n.a.	Invasive
Myriophyllum heterophyllum Michx.	twoleaf watermilfoil	M	Y	Y	Y	n.a.	Invasive
Najas graminea Delile	ricefield waternymph	M	–	n.e.	–	N	Non-invasive
Nymphaea candida C.Presl	–	M	–	n.e.	–	N	Non-invasive
Pistia stratiotes L.	water lettuce	P	Y	Y	–	n.a.	Invasive
Horizon							
Cabomba caroliniana A.Gray	Carolina fanwort	–	Y	Y	–	n.a.	Invasive
Gymnocoronis spilanthoides (D.Don ex Hook. & Arn.) DC.	Senegal tea plant	–	Y	Y	–	n.a.	Invasive
Hygrophila polysperma (Roxb.) T.Anderson	Indian swampweed	–	Y	Y	–	n.a.	Invasive
Lemna aequinoctialis Welw.	lesser duckweed	–	–	N	–	N	Non-invasive
Lemna minuta Kunth	least duckweed	–	–	Y	–	n.a.	Invasive
Lemna turionifera Landolt	turion duckweed	–	–	–	–	N	Non-invasive
Najas guadalupensis (Spreng.) Magnus	southern waternymph	–	–	n.e.	–	N	Non-invasive
Nelumbo nucifera Gaertn.	sacred lotus	–	–	n.e.	–	N	Non-invasive
Nymphaea lotus L.	white Egyptian lotus	–	–	N	–	N	Non-invasive
Rotala macrandra Koehne	–	–	–	N	–	N	Non-invasive
Rotala rotundifolia (Buch.-Ham. ex Roxb.) Koehne	dwarf rotala	–	–	–	Y	n.a.	Invasive
Sagittaria subulata (L.) Buchenau	awl-leaf arrowhead	–	–	–	–	N	Non-invasive
Utricularia gibba L.	humped bladderwort	–	Y	N	–	n.a.	Invasive
Vallisneria australis S.W.L.Jacobs & Les	–	–	–	–	–	N	Non-invasive

(http://www.worldfloraonline.org/) and The PLANTS Database (https://plants.usda.gov/home). Selection of emergent, submergent or floating aquatic plants was based on two criteria: (i) extant species, i.e. already present in both risk assessment areas (n = 10) and identified using the MINGOR 2022 (https://invazivnevrste.haop.hr/katalog) and Flora Croatica (http://hirc.botanic.hr/fcd) databases; and (ii) horizon species, i.e. likely to enter the risk assessment areas in the near future (n = 14) and identified with the aid of the Centre for Agriculture and Bioscience International (CABI) scanning tool (www.cabi.org/horizonscanningtool). Screenings were conducted independently by authors TR, MP and MB for both risk assessment areas, with each assessor screening a subset of the total number of species (i.e. nine, eight and seven species, respectively). Notably, the screening of a set of species for a certain risk assessment area by more than one independent assessor has been shown to provide no advantage in terms of increased level of confidence compared to screenings carried out by the same assessors on subsets of the total number of species. This is the approach followed in the present study, which has important implications in terms of allocation of resources and cost-benefit analysis (combination 1IS: Vilizzi et al. 2022).

Risk identification was undertaken using the Aquatic Species Invasiveness Screening Kit (AS-ISK: Copp et al. 2016, 2021), which is available for free download at www.cefas.co.uk/nns/tools/). This taxon-generic decision-support tool complies with the 'minimum standards' for screening non-native species under EC Regulation No. 1143/2014 on the prevention and management of the introduction and spread of invasive alien species (EU 2014). The AS-ISK consists of 55 questions: the first 49 questions comprise the Basic Risk Assessment (BRA) and address the biogeography/invasion history and biology/ecology of the species; the last six questions comprise the Climate Change Assessment (CCA) and require the assessor to predict how future predicted climatic conditions are likely to affect the BRA with respect to risks of introduction, establishment, dispersal and impact. The BRA questions consist of two sections with eight categories: Section A of Biogeography/Invasion History including the categories Domestication/Cultivation (C1), Climate, distribution and introduction risk (C2) and Invasive elsewhere (C3); Section B of Biology/Ecology including the categories Undesirable (or persistence) traits (C4), Resource exploitation (C5), Reproduction (C6), Dispersal mechanisms (C7) and Tolerance attributes (C8); Section C of Climate change including the (implicit) category Climate change (C9) (see Suppl. material 1: Table S1).

To achieve a valid screening, the assessor must provide for each question a response, a confidence level for the response (see below) and a justification, based on literature sources. The outcomes are a BRA score and a (composite) BRA+CCA score, which is obtained after adding or subtracting up to 12 points to the BRA score or leaving it unchanged in case of a CCA score equal to 0. Scores < 1 suggest that the species poses a 'low risk' to become invasive in the risk assessment area, whereas scores ≥ 1 indicate a 'medium risk' or a 'high risk'. The threshold (Thr) value to distinguish between medium-risk (BRA and BRA+CCA score < Thr) and high-risk (BRA and BRA+CCA score ≥ Thr) species for the risk assessment area is obtained by 'calibration', based on the Receiver Operating Characteristic (ROC) curve analysis (see Vilizzi et al. 2022). A

measure of the accuracy of the calibration analysis is the area under the curve (AUC) whose values are interpreted as: $0.7 \leq \text{AUC} < 0.8$ = acceptable discriminatory power, $0.8 \leq \text{AUC} < 0.9$ = excellent, $0.9 \leq \text{AUC}$ = outstanding (Hosmer et al. 2013). For the species classified as high risk, a distinction was made in this study of the 'very high risk' species, based on an *ad hoc* threshold, weighted according to the range of high-risk score values obtained for the BRA and BRA+CCA. Identification of the (very) high-risk species is useful to prioritise allocation of resources in view of a full risk assessment (Copp et al. 2016). This examines in detail the risks of: (i) introduction (entry); (ii) establishment (of one or more self-sustaining populations); (iii) dispersal (more widely within the risk assessment area, i.e. so-called secondary spread or introductions); and (iv) impacts (to native biodiversity, ecosystem function and services and the introduction and transmission of diseases).

For ROC curve analysis to be implemented, the species selected for screening must be categorised *a priori* as 'non-invasive' or 'invasive' using literature sources. The *a priori* categorisation of species was implemented as per Vilizzi et al. (2022) (Table 1). Confidence levels in the responses to questions in the AS-ISK are ranked using a 1–4 scale and, based on the confidence level (CL) allocated to each response, a confidence factor (CF) is obtained as:

$$\text{CF} = \sum(\text{CL}_{\text{Q}i})/(4 \times 55) \ (i = 1, \ldots, 55)$$

where $\text{CL}_{\text{Q}i}$ is the CL for Q*i*, 4 is the maximum achievable value for confidence (i.e. very high: see above) and 55 is the total number of questions comprising the AS-ISK questionnaire (Vilizzi et al. 2022). The CF ranges from a minimum of 0.25 (i.e. all 55 questions with confidence level equal to 1) to a maximum of 1 (i.e. all 55 questions with confidence level equal to 4). Based on all 55 Qs of the AS-ISK questionnaire, the 49 Qs comprising the BRA and the six Qs comprising the CCA, the CF_{Total}, CF_{BRA} and CF_{CCA} are respectively computed.

Implementation of ROC curve analysis followed the protocol described in Vilizzi et al. (2022), with the true/false positive/negative outcome distinction not applied to the medium-risk species, as they can be either included or not into a full (comprehensive) risk assessment depending on priority and/or availability of financial resources. Following ROC analysis, the best threshold value that maximises the true positive rate and minimises the false positive rate was determined using Youden's *J* statistic, whereas the 'default' threshold of 1 was set to distinguish between low-risk and medium-risk species. Fitting of the ROC curve was with package pROC (Robin et al. 2011) for R ×64 v.4.0.5 (R Core Team 2021) using 2000 bootstrap replicates for the confidence intervals of specificities, which were computed along the entire range of sensitivity points (i.e. 0 to 1, at 0.1 intervals). Differences in mean BRA and BRA+CCA scores between risk assessment areas (Pannonian Region, Mediterranean Region) were statistically tested with permutational ANOVA, based on a one-factor design. Differences in CF between risk assessment areas, components (BRA, BRA+CCA) and species status (Extant, Ho-

rizon) were also tested with permutational ANOVA, based on a nested-factorial design with factors risk assessment area and Component crossed and factor Status nested within Risk assessment area × Component and with all factors fixed. Analysis was implemented in PERMANOVA+ for PRIMER v.7, with normalisation of the data and using a Bray-Curtis dissimilarity measure, 9999 permutations of the raw data (unrestricted in case of the factorial-nested design) and with statistical effects evaluated at α = 0.05.

Results

For the Pannonian Region: the BRA scores ranged from 5.5 to 41.0, with mean = 23.6, median = 22.8 and 5% and 95% CI = 6.3 and 39.9; the BRA+CCA scores ranged from 5.5 to 53.0, with mean = 31.5, median = 32.5 and 5% and 95% CI = 8.4 and 51.9. For the Mediterranean region: the BRA scores ranged from 6.0 to 41.0, with mean = 25.5, median = 32.3 and 5% and 95% CI (confidence interval) = 6.7 and 40.0; the BRA+CCA scores ranged from 6.0 to 53.0, with mean = 32.5, median = 35.8 and 5% and 95% CI = 8.4 and 51.0. There were no differences in the mean BRA scores for the Pannonian and Mediterranean regions ($F^{\#}_{1,46}$ = 0.245, P = 0.640; # = permutational value) nor in the mean BRA+CCA scores ($F^{\#}_{1,46}$ = 0.055, P = 0.816).

Risk outcomes

The ROC curve for the Pannonian Region resulted in an AUC of 0.8357 (0.6410–1.0000 95% CI) and for the Mediterranean Region in an AUC of 0.8679 (0.6864–1.0000 95% CI). Both AUCs had, therefore, excellent discriminatory power, hence were able to classify reliably non-invasive and invasive aquatic plant species for the two risk assessment areas. Youden's *J* provided the thresholds of 22.75 and 24.75 for the Pannonian and Mediterranean regions, respectively. These thresholds were used for calibration of the risk outcomes to distinguish between medium-risk and high-risk species (combined AS-ISK report in Suppl. material 2).

For the Pannonian Region (Table 2):

- Based on the BRA outcome scores: 12 (50.0%) species were classified as high risk and 12 (50.0%) as medium risk. Amongst the 14 species categorised *a priori* as invasive, eleven were true positives and amongst the 10 species categorised *a priori* as non-invasive, one was a false positive. Of the 12 medium-risk species, nine were *a priori* non-invasive and three invasive.
- Based on the BRA+CCA outcome scores (hence, after accounting for climate change predictions): 18 (75.0%) species were classified as high risk and six (25.0%) as medium risk. Amongst the *a priori* invasive species, twelve were true positives and amongst the *a priori* non-invasive species, six were false positive. Of the six medium-risk species, four were *a priori* non-invasive and two invasive.

Table 2. Risk outcomes for the non-native aquatic plant species screened with the Aquatic Species Invasiveness Screening Kit (AS-ISK) for the Pannonian and Mediterranean regions of Croatia. For each species, the following information is provided: *a priori* categorisation of invasiveness (N = non-invasive; Y = invasive: see Table 1); BRA and BRA+CCA scores with corresponding risk outcomes (M = Medium; H = High; VH = Very high, based on an *ad hoc* threshold equal to 40: see text for details) and classification (Class: FP = false positive; TP = true positive; '–' = not applicable as medium-risk: see text for details); difference (Delta) between BRA+CCA and BRA scores; confidence factor (CF) for all 55 questions of the AS-ISK (CF_{Total}), for the 49 BRA questions (CF_{BRA}) and for the six CCA questions (CF_{CCA}). Risk outcomes are based on the thresholds (Thr) of 22.75 for the Pannonian Region and 24.75 for the Mediterranean Region. Risk outcomes for the BRA are computed as: M, within the interval [1, Thr, H [Thr, 40[and VH [40, 68]. Risk outcomes for the BRA+CCA are computed as: M [1, Thr, H [Thr, 40[and VH [40, 68] (note the reverse bracket notation indicating in all cases an open interval).

Species name	A priori	BRA			BRA+CCA				CF		
		Score	Outcome	Class	Score	Outcome	Class	Delta	Total	BRA	CCA
Pannonian Region											
Azolla cristata	Y	33.0	H	TP	45.0	VH	TP	12.0	0.53	0.51	0.67
Azolla filiculoides	Y	30.0	H	TP	42.0	VH	TP	12.0	0.66	0.67	0.63
Cabomba caroliniana	Y	23.0	H	TP	23.0	H	TP	0.0	0.48	0.48	0.50
Egeria densa	Y	36.0	H	TP	48.0	VH	TP	12.0	0.57	0.58	0.50
Elodea canadensis	Y	39.0	H	TP	51.0	VH	TP	12.0	0.68	0.68	0.63
Elodea nuttallii	Y	41.0	VH	TP	53.0	VH	TP	12.0	0.68	0.67	0.75
Gymnocoronis spilanthoides	Y	28.0	H	TP	40.0	VH	TP	12.0	0.68	0.65	0.92
Hygrophila polysperma	Y	35.5	H	TP	47.5	VH	TP	12.0	0.72	0.73	0.63
Lemna aequinoctialis	N	40.0	VH	FP	52.0	VH	FP	12.0	0.72	0.72	0.71
Lemna minuta	Y	33.0	H	TP	43.0	VH	TP	10.0	0.71	0.73	0.50
Lemna turionifera	N	21.0	M	–	27.0	H	FP	6.0	0.71	0.73	0.58
Ludwigia peploides	Y	22.0	M	–	22.0	M	–	0.0	0.47	0.46	0.58
Myriophyllum heterophyllum	Y	24.0	H	TP	34.0	H	TP	10.0	0.53	0.52	0.58
Najas graminea	N	11.5	M	–	11.5	M	–	0.0	0.42	0.41	0.50
Najas guadalupensis	N	17.0	M	–	29.0	H	FP	12.0	0.48	0.48	0.50
Nelumbo nucifera	N	19.0	M	–	31.0	H	FP	12.0	0.54	0.57	0.29
Nymphaea candida	N	5.5	M	–	5.5	M	–	0.0	0.40	0.38	0.58
Nymphaea lotus	N	14.5	M	–	26.5	H	FP	12.0	0.68	0.69	0.58
Pistia stratiotes	Y	15.0	M	–	23.0	H	TP	8.0	0.53	0.53	0.54
Rotala macrandra	N	8.0	M	–	8.0	M	–	0.0	0.41	0.40	0.50
Rotala rotundifolia	Y	14.0	M	–	14.0	M	–	0.0	0.50	0.51	0.50
Sagittaria subulata	N	6.0	M	–	6.0	M	–	0.0	0.37	0.36	0.50
Utricularia gibba	Y	28.5	H	TP	40.5	VH	TP	12.0	0.65	0.66	0.54
Vallisneria australis	N	22.5	M	–	34.5	H	FP	12.0	0.61	0.61	0.63
Mediterranean Region											
Azolla cristata	Y	32.0	H	TP	44.0	VH	TP	12.0	0.52	0.51	0.63
Azolla filiculoides	Y	39.0	H	TP	51.0	VH	TP	12.0	0.67	0.67	0.63
Cabomba caroliniana	Y	29.5	H	TP	39.5	H	TP	10.0	0.62	0.63	0.58
Egeria densa	Y	36.0	H	TP	48.0	VH	TP	12.0	0.57	0.58	0.50
Elodea canadensis	Y	39.0	H	TP	51.0	VH	TP	12.0	0.68	0.69	0.63
Elodea nuttallii	Y	41.0	VH	TP	53.0	VH	TP	12.0	0.68	0.67	0.75
Gymnocoronis spilanthoides	Y	28.0	H	TP	38.0	H	TP	10.0	0.65	0.63	0.75

Species name	*A priori*	BRA			BRA+CCA				CF		
		Score	Outcome	Class	Score	Outcome	Class	Delta	Total	BRA	CCA
Hygrophila polysperma	Y	40.0	VH	TP	44.0	VH	TP	4.0	0.69	0.70	0.58
Lemna aequinoctialis	N	40.0	VH	FP	48.0	VH	FP	8.0	0.70	0.72	0.46
Lemna minuta	Y	33.0	H	TP	33.0	H	TP	0.0	0.75	0.78	0.50
Lemna turionifera	N	20.0	M	–	26.0	H	FP	6.0	0.70	0.72	0.54
Ludwigia peploides	Y	26.5	H	TP	36.5	H	TP	10.0	0.52	0.52	0.54
Myriophyllum heterophyllum	Y	27.5	H	TP	37.5	H	TP	10.0	0.53	0.52	0.58
Najas graminea	N	14.5	M	–	14.5	M	–	0.0	0.45	0.44	0.50
Najas guadalupensis	N	13.5	M	–	25.5	H	FP	12.0	0.46	0.44	0.58
Nelumbo nucifera	N	23.0	M	–	35.0	H	FP	12.0	0.54	0.57	0.29
Nymphaea candida	N	6.5	M	–	10.5	M	–	4.0	0.37	0.37	0.38
Nymphaea lotus	N	14.5	M	–	24.5	M	–	10.0	0.67	0.69	0.46
Pistia stratiotes	Y	18.0	M	–	18.0	M	–	0.0	0.51	0.51	0.50
Rotala macrandra	N	8.0	M	–	8.0	M	–	0.0	0.40	0.39	0.50
Rotala rotundifolia	Y	16.0	M	–	16.0	M	–	0.0	0.50	0.50	0.50
Sagittaria subulata	N	6.0	M	–	6.0	M	–	0.0	0.36	0.34	0.50
Utricularia gibba	Y	32.0	H	TP	42.0	VH	TP	10.0	0.65	0.66	0.54
Vallisneria australis	N	21.5	M	–	31.5	H	FP	10.0	0.60	0.59	0.67

The highest-scoring species (BRA and BRA+CCA scores ≥ 40, taken as an *ad hoc* 'very high risk' threshold) were *Elodea nuttallii* and *Lemna aequinoctialis* and, after accounting for the CCA, also *Elodea canadensis*, *Egeria densa*, *Hygrophila polysperma*, *Azolla cristata*, *Lemna minuta*, *Azolla filiculoides*, *Utricularia gibba* and *Gymnocoronis spilanthoides*. The CCA resulted in an increase in the BRA score (cf. BRA+CCA score) for 17 species and in no change for the remaining seven (Table 2).

For the BRA score outcomes, there were discrepancies in risk ranking between the two risk assessment areas only for *Ludwigia peploides*, which was high risk for the Mediterranean Region, but medium risk for the Pannonian Region (Fig. 2A). For the BRA+CCA score outcomes, there were discrepancies for *Cabomba caroliniana*, *Ludwigia peploides* and *Myriophyllum heterophyllum*, which were high risk for the Mediterranean Region and medium risk for the Pannonian Region and for *Lemna minuta*, which was medium risk for the Mediterranean Region and high risk for the Pannonian Region (Fig. 2B).

For the Mediterranean Region (Table 2):

- Based on the BRA outcome scores: 13 (54.2%) species were classified as high risk and 11 (45.8%) as medium risk. Amongst the 14 species categorised *a priori* as invasive, 12 were true positives and, amongst the 10 species categorised *a priori* as non-invasive, one was a false positive. Of the eleven medium-risk species, nine were *a priori* non-invasive and two invasive.
- Based on the BRA+CCA outcome scores (hence, after accounting for climate change predictions): 17 (70.8%) species were classified as high risk and seven (29.2%) as medium risk. Amongst the *a priori* invasive species, 12 were true positives and amongst the *a priori* non-invasive species, five were false positives. Of the seven medium-risk species, five were *a priori* non-invasive and two invasive.

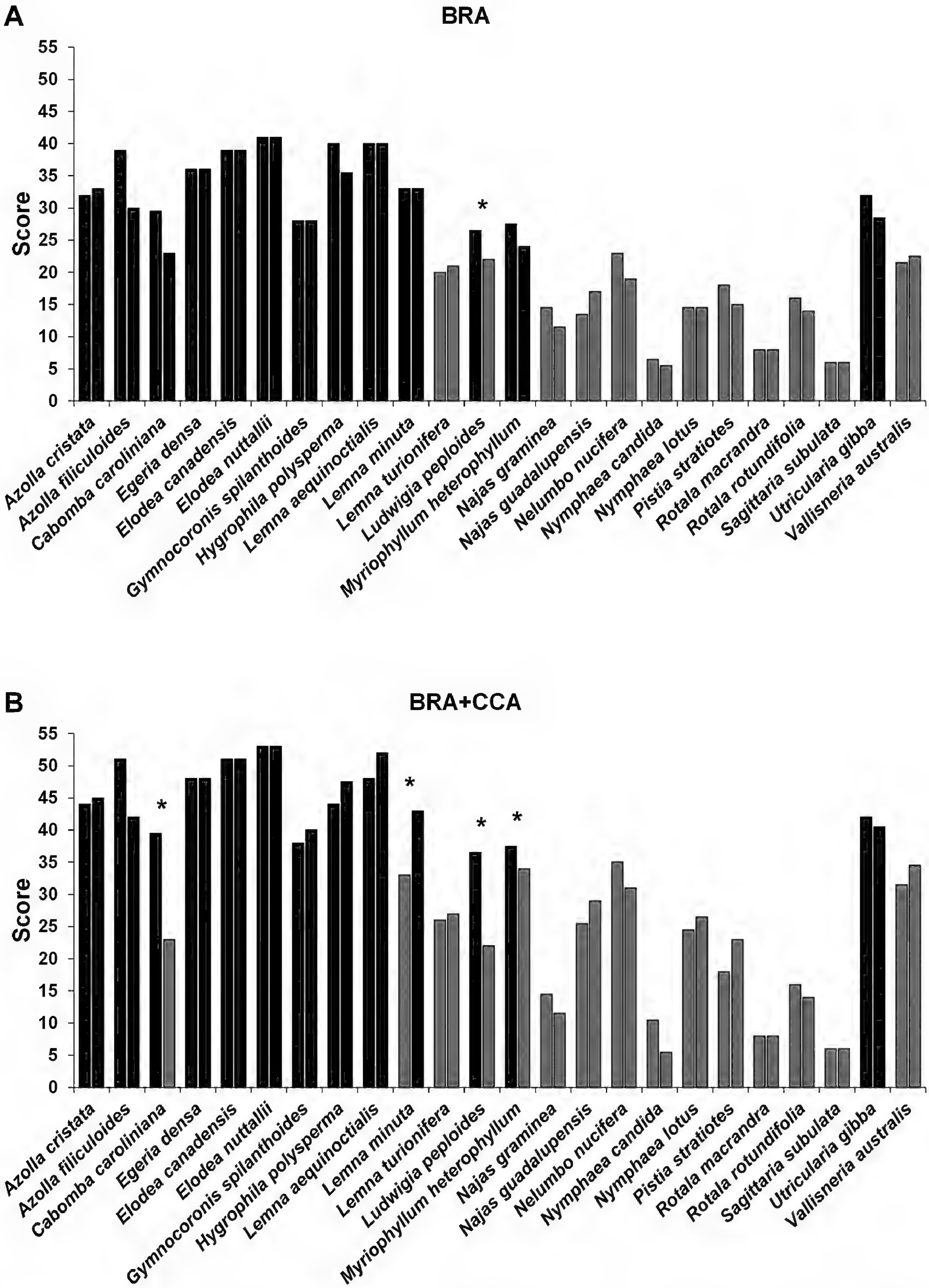

Figure 2. A Basic Risk Assessment (BRA) scores for the non-native aquatic plants screened for their risk of invasiveness in the Pannonian (right bars) and Mediterranean (left bars) regions of Croatia **B** same for the BRA + CCA (Climate Change Assessment) scores. Grey bars = medium risk; black bars = high (or very high) risk (see Table 2). Asterisk indicates species with different risk outcome for the two risk assessment areas.

The highest-scoring species (same very high-risk threshold as for the Pannonian Region) were *Elodea nuttallii*, *Hygrophila polysperma* and *Lemna aequinoctialis* and, after accounting for the CCA, also *Azolla filiculoides*, *Elodea canadensis*, *Egeria densa*, *Azolla cristata* and *Utricularia gibba*. The CCA resulted in an increase in the BRA score (cf. BRA+CCA score) for 18 species and in no change for the remaining six (Table 2).

Confidence and discrepancies in responses

For the Pannonian Region, the mean CF_{Total} was 0.573 ± 0.023 SE, the mean CF_{BRA} 0.573 ± 0.025 SE and the mean CF_{CCA} 0.576 ± 0.024 SE. For the Mediterranean Region, the mean CF_{Total} was 0.573 ± 0.023 SE, the mean CF_{BRA} 0.577 ± 0.025 SE and the mean CF_{CCA} 0.545 ± 0.021 SE. There were no differences in mean CF between risk assessment areas, Components and Status within Risk assessment area × Component (Table 3).

Most discrepancies in the responses, as measured by the number of species for which a different response was provided to a certain question (Q), were for all the Climate, distribution and introduction risk and Climate change questions. There were also discrepancies for four of the 12 Qs related to Undesirable (or persistence) traits, as well as for one Q in each of the Resource exploitation, Reproduction and Dispersal mechanisms sections (Fig. 3).

Table 3. Permutational ANOVA results for the confidence factor (CF) of the non-native aquatic plant species risk screened for the Pannonian and Mediterranean regions of Croatia – the risk assessment areas. Component = BRA, BRA+CCA (see Table 2); Status = Extant, Horizon (see Table 1); # = permutational value.

Source of variation	df	MS	$F^{\#}/t$	$P^{\#}$
Risk assessment area	1	0.326	0.393	0.540
Component	1	0.089	0.108	0.658
Risk assessment area × Component	1	0.562	0.677	0.467
Status (Risk assessment area × Component)	4	0.831	0.808	0.518
Residual	88	1.027		

Discussion

This study is the first calibrated application of the AS-ISK on aquatic plants for a defined risk assessment area (see Vilizzi et al. 2021). Classification of the screened aquatic plant species into medium-risk and high-risk was successfully achieved for the Pannonian and Mediterranean regions of Croatia with a high degree of accuracy (as indicated by the excellent discriminatory power) and the threshold values of 22.75 for the Pannonian Region and 24.75 for the Mediterranean Region are overall in accordance with the global value of 24.5 obtained for freshwater aquatic plants (Vilizzi et al. 2021).

The top invasive species ranked as very high-risk under both current and predicted climate conditions in both risk assessment areas were *Elodea nuttallii* and

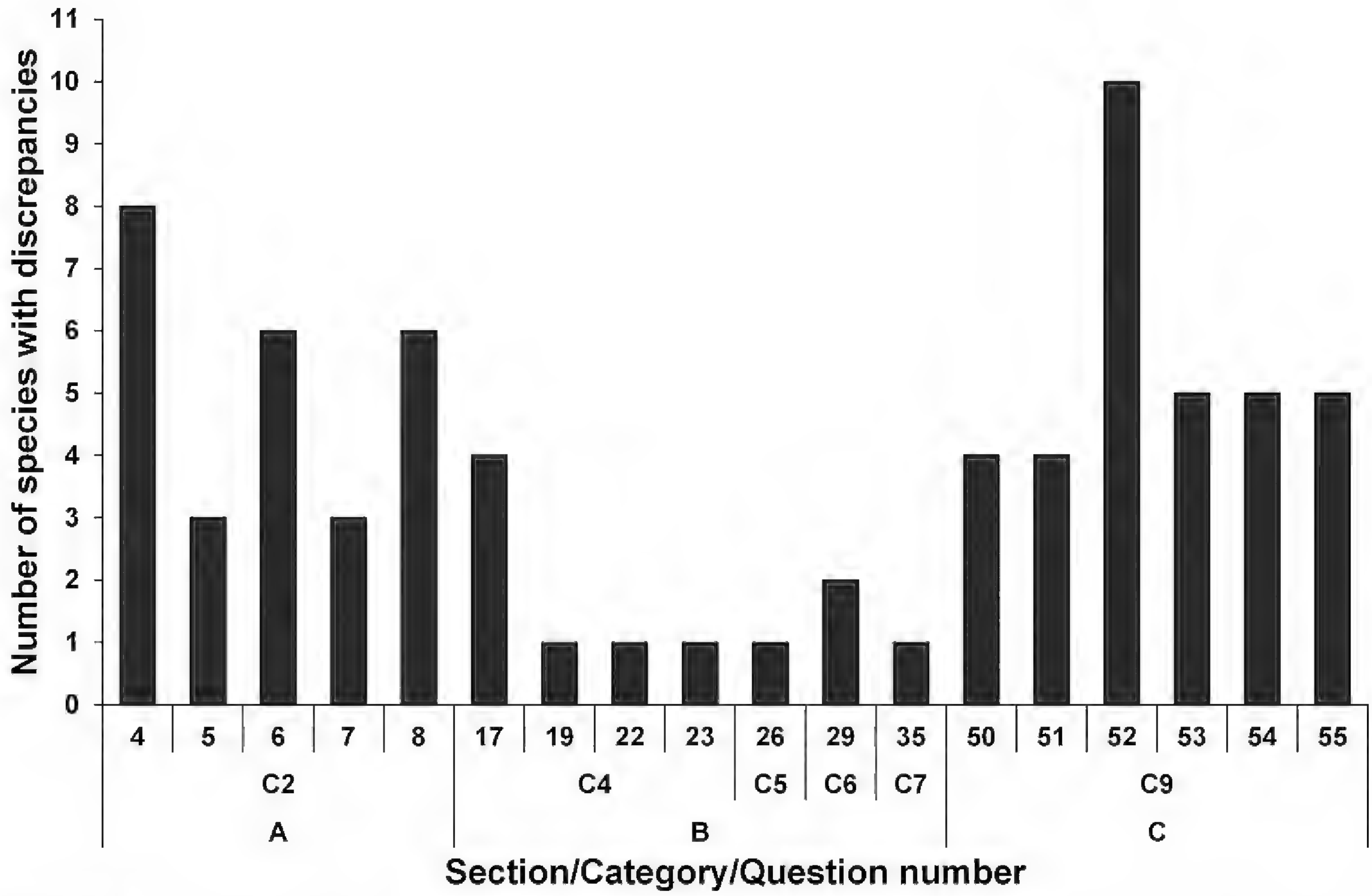

Figure 3. Number of species for which discrepancies in the responses to the AS-ISK questions were found, based on screening for the Pannonian vs. the Mediterranean regions. Section: A = Biogeography/Invasion history; B = Domestication/Cultivation; C = Climate change. Category: C2 = Climate, distribution and introduction risk; C4 = Undesirable (or persistence) traits; C5 = Resource exploitation; C6 = Reproduction; C7 = Dispersal mechanisms; C9 = Climate change. Question codes as per Suppl. material 1: Table S1.

Lemna aequinoctialis. *Elodea nuttallii* is a perennial submerged aquatic plant native to North America and one of the most widespread non-native species in Europe (Hussner 2012) and is found in the Pannonian Region near the Hungarian border (Király et al. 2007; Kočić et al. 2014). Although this species has not yet been recorded in the Mediterranean Region (Nikolić 2022), it is known to be established in its proximity. *Elodea nuttallii* was accidentally introduced as an aquarium plant into Europe, where it was first recorded at the beginning of the 20th century (Cook and Urmi-König 1985). This species has since become widespread throughout the continent (Steen et al. 2019) and, since 2017, has been included in the List of Invasive Alien Species of Union concern (European Union Regulation 1143/2014: EU 2014). The reason for its successful colonisation is attributable to its vegetative reproduction by fragmentation, stem division and production of winter buds as the dominant method of propagation (Thiébaut and Di Nino 2009). In addition, *E. nuttallii* has wide tolerance of habitats and salinity, benefits from anthropogenic pressure, is characterised by vigorous growth (Steen et al. 2019) and is less affected by water temperature fluctuations than native aquatic plants (Fritz et al. 2017). The climate conditions in the Pannonian Region match the species' invaded habitats of Europe, hence rapid spread and colonisation

are highly likely under both climate scenarios (Rodríguez-Merino et al. 2018). Although *E. nuttallii* reproduces by vegetative propagation in warm waters (≈ 20 °C) (Hoffmann et al. 2015), it has a temperature limit (Netten et al. 2010). However, as the Mediterranean Region belongs to the Dinaric karst, most of its water bodies are short and their mean annual temperature does not exceed 13 °C (Horvatinčić et al. 2003; Bonacci and Roje-Bonacci 2012; Bonacci et al. 2014); hence, under predicted global warming, the environmental conditions of freshwater ecosystems in this area may become even more suitable for this species (Rodríguez-Merino et al. 2018).

Lemna aequinoctialis is a horizon species for both risk assessment areas that has a broad distribution extending over several continents and has expanded its range to become cosmopolitan (Crawford et al. 2006; Tippery and Les 2020). Although *L. aequinoctialis* is not yet naturalised in most European countries where it is recorded (Hussner et al. 2010), it has high potential to become a new component of European aquatic ecosystems (Crawford et al. 2006). Growth rate of *L. aequinoctialis* under optimal conditions is close to exponential and its frond number may almost double within 24 h, making it one of the fastest-growing flowering plants (Fourounjian et al. 2020). This species can tolerate extreme ranges in pH from 3.2 to more than 9.0 (Thiébaut 2007) and can produce turions (dormant vegetative organs) in response to unfavourable environmental conditions (i.e. decreased temperature, day length or nutrient availability) (Fourounjian et al. 2020; Tippery and Les 2020). The turions overwinter on the bottom water in darkness under hypoxic or anoxic conditions and resume growth once water temperature reaches about 15 °C (Fourounjian et al. 2020; Schweingruber et al. 2020). Despite evidence that *L. aequinoctialis* cannot tolerate temperatures below 0 °C (Vélez-Gavilán 2017), this species seems already naturalised in the Pannonian Region of Hungary (Lukács et al. 2014) and has been reported from France, Germany, Greece and Sweden (Ryman and Anderberg 1999; Thiébaut 2007; Hussner et al. 2010; Lansdown et al. 2016). The climate of the Mediterranean Region under current conditions fully matches the requirements of *L. aequinoctialis* (Vélez-Gavilán 2017), though an increase in mean annual temperatures in both risk assessment areas and particularly in the Pannonian Region, may expedite the species' naturalisation process (Beck et al. 2018; Rodríguez-Merino et al. 2018). Importantly, as *L. aequinoctialis* can be misidentified with native or introduced *Lemna* sp. (Xu et al. 2015), it is highly likely to pose a greater risk of invasiveness than previously assumed.

The horizon species *Hygrophila polysperma* ranked as very high-risk for the Mediterranean Region under current climate conditions and in both risk assessment areas under projected climate conditions. This species naturally occurs in tropical Asia, India and Malaysia and was introduced to Florida and Texas (USA), where it is established (Angerstein and Lemke 1994). However, *H. polysperma* has also recently been found in Europe (i.e. Austria, Germany, Hungary and Poland) in thermally heated waters, where it was probably released from aquaria (Hussner 2012; EPPO 2017). This species has been flagged as high priority in the list of the European Union pest risk analysis (Tanner et al. 2017) due to its high phenotypic plasticity, tolerance of a wide range of habitats, predominant spread via fragments with high regeneration rates and build-up

of high biomass densities, which cause it to occupy the entire water column and outcompete by shading with both native and other invasive plant species (EPPO 2017). Under current climate conditions, the Mediterranean coastline of the risk assessment area may be suitable for the naturalisation of *H. polysperma*, whereas under a scenario of climate change, potentially suitable regions for colonisation are the Continental, Black Sea and Atlantic biogeographical regions (EPPO 2017).

Extant *Azolla cristata*, *Azolla filiculoides*, *Elodea canadensis*, *Egeria densa* and horizon *Utricularia gibba* were ranked as high-risk under current climate conditions and, after accounting for climate change, they became very high-risk for both risk assessment areas, whereas horizon *Gymnocoronis spilanthoides* and *Lemna minuta* were ranked as very high-risk under climate change only for the Pannonian Region. *Azolla filiculoides* and *E. canadensis* are the most widespread non-native aquatic plants in Europe (Hussner 2012). The native distributional range of *A. cristata* and *A. filiculoides* extends from North to South America, reflecting their wide temperature and climate tolerance (Troy et al. 2022), whereas *E. canadensis* originates from North America, with most European climates matching its ecological requirements (Duenas-Lopez et al. 2018).

Azolla filiculoides was recorded for the first time in Kopački Rit in 1978 and *Azolla cristata* in 1982 from Osijek in a hydromelioration channel and at Vukovar town in backwaters near the River Danube (Trinajstić and Pavletić 1978; Nikolić 2022). Further records of *Azolla* spp. in Croatia have been related to *A. filiculoides*, which is distributed along the River Drava (Nikolić 2022). However, difficulties in distinguishing between *Azolla* species have led to a long history of misidentifications and taxonomic confusion (Reid et al. 2006). As a result, the finding of *A. cristata* may be either a misidentification for Croatia or its replacement (i.e. by competition/overgrowth) by *A. filiculoides* due to its greater adaptability to eutrophication caused by urbanisation and agricultural activities (Lastrucci et al. 2019). Indeed, in Serbia, which borders Croatia through the River Danube, only *A. filiculoides* has been recognised as present in the aquatic systems of the region (Anđelković et al. 2016). In addition, a survey performed in Czechia revealed that only *A. filiculoides* is present and that *A. cristata* has never occurred in the country (Pyšek et al. 2012). Similar findings have been reported from Portugal (Pereira et al. 2001) and Italy, where *A. cristata* has disappeared from the wild (Lastrucci et al. 2019), with the only reliable record of *A. cristata* in Europe having been confirmed for The Netherlands (Pyšek et al. 2012). Current climate conditions for both risk assessment areas seem suitable for both *Azolla* species (Peel et al. 2007). Nevertheless, *A. filiculoides* under climate change scenarios will extend its distribution northwards and at higher altitudes, so that part of the Mediterranean habitats may become unsuitable (Rodríguez-Merino et al. 2019). However, the boreal climate type in the mountains of the Mediterranean Region of Croatia matches the climate types of northern Europe, including those of the European Alps under both scenarios (Peel et al. 2007; Rubel et al. 2017), making these regions particularly suitable for invasion. On the contrary, *A. cristata* seems to tolerate a higher thermal range (Madeira et al. 2016); hence, under a climate change scenario, this species is likely to adapt to the southern part of the Mediterranean Region.

Elodea canadensis has a long history of establishment in Croatia and, similar to its congener *Elodea nuttallii*, is perennial, has wide ecological tolerance with overwintering in deeper waters, asexual reproduction and relatively fast growth (Barrat-Segretain et al. 2002). *Elodea canadensis* is currently well distributed across the Pannonian Region, but not yet recorded in the Mediterranean Region (Kočić et al. 2014). However, due to its long history of establishment in Croatia and several aquatic species translocations (Pofuk et al. 2017), *E. canadensis* may also have been transferred, but overlooked, particularly because of the reported lack of a full inventory of aquatic plants in the Mediterranean Region. Under climate change predictions, suitable areas for *E. canadensis* extend into several Mediterranean countries and areas next to the Black Sea (Heikkinen et al. 2009), hence making this species highly likely to become more invasive in Croatia.

The native distribution of *Egeria densa* is temperate and sub-tropical South America, whereas the distribution of *Gymnocoronis spilanthoides* and *Lemna minuta* extends to tropical regions of North and South America. *Utricularia gibba* has a mostly pantropical distribution and, apart from North America, occurs in Asia, the Pacific and the western Mediterranean (CABI 2022). All four species have been introduced around the world primarily by the aquarium and pet trade (Saul et al. 2017). Amongst these species, naturalised populations of *E. densa* were recently recorded in the Mediterranean Region of Croatia in the River Neretva Basin in clear, slow flowing, oligohaline waters with high alkalinity and conductivity, where the species surpasses in abundance the indigenous ones (Rimac et al. 2018). The presence of only male specimens of *E. densa* has been observed in Croatia, similar to other countries' part of the species' introduced range of distribution (Thiébaut et al. 2016; Rimac et al. 2018). The principal means of *E. densa* reproduction is vegetatively by fragmentation of stems, which can form dense mats (Thiébaut et al. 2016). This species tolerates a wide range of climate types that overlap with the climates of both risk assessment areas and, under predicted climate change conditions, it is likely to remain as high-risk particularly at higher altitudes of the risk assessment areas (Gillard et al. 2017). In proximity to the risk assessment areas, *G. spilanthoides* and *L. minuta* are found in Hungary, whereas *U. gibba* has also been recorded in Serbia (Hussner 2012; CABI 2022). *Utricularia gibba* is an annual or perennial submerged or free-floating carnivorous aquatic plant that can rapidly colonise new water bodies by stem fragmentation or by its seeds forming a dense mat cover on the water surface (deWinton et al. 2009; CABI 2022). Considering its wide distribution and tolerance of a wide range of temperatures and habitats, this species may become a serious threat under both current and projected climate conditions in the risk assessment areas. *Gymnocoronis spilanthoides* is naturalised in Europe, grows very rapidly, easily reproduces vegetatively by any parts of its stem, forms floating mats that may cover entire water bodies, blocks drainage channels and degrades natural wetlands by displacing native plants and animals – all of these traits make it of higher threat for Europe than initially predicted (Ardenghi et al. 2016). *Lemna minuta* is a small free-floating plant that also forms dense mats on the water surface, reduces light penetration and gas exchange, causes the disappearance of submersed aquatic plants and alters invertebrate community composition and abundance (Ceschin et al. 2020).

This species is established and widespread in several countries and climate types in Europe (Paolacci et al. 2018) but has not yet been recorded in the risk assessment areas. However, *L. minuta* can be easily overlooked and confused with native *Lemna minor*. This is because the only reliable diagnostic character is the vein number, which is not easy to identify in the field: *L. minuta* has just one, whereas *L. minor* has three (Bog et al. 2010; Gérard and Triest 2018). Under current climate conditions, *G. spilanthoides* and *L. minuta* gained a high risk of invasiveness but may become very high-risk in the Pannonian Region likely due to the availability of habitats consisting of slow-flowing or lentic water bodies and wet–marshy soils and wetlands, which are not well represented in the Mediterranean Region.

In both risk assessment areas, *Cabomba caroliniana* and *Myriophyllum heterophyllum* were ranked as high-risk under both climate scenarios, whereas *Ludwigia peploides* was high-risk under both climate scenarios in the Pannonian Region only. The native distribution of *C. caroliniana* covers the eastern part of subtropical and temperate areas of South America (Roberts and Florentine 2022), hence matching current and projected climate conditions in both risk assessment areas (Beck et al. 2018; Rodríguez-Merino et al. 2018). This species is established in Serbia (Vojvodina, near the Croatian border), where it has expanded from Hungary probably through the canal network of the hydrosystem Danube-Tisa-Danube (Anđelković et al. 2016). In Europe, *C. caroliniana* is still not regarded as invasive and is mostly found in localised and scattered populations (Roberts and Florentine 2022), though in The Netherlands, it has been declared as high risk (Matthews et al. 2013). The species' high invasiveness has been reported in its non-native distributional range, primarily due to its high competitiveness, dense and persistent growth, asexual reproduction through stem auto-fragmentation and tolerance of extreme pH ranges from 4.0 to 8.8 (Matthews et al. 2013). The species' population expansion in connected waterways, as occurring in the Pannonian Region, may be facilitated by its long fragments that can get wrapped in boat motors, boating or anglers' equipment (Roberts and Florentine 2022). In addition, vectors like fish re-stocking and spread by birds cannot be ruled out as potential pathways of introduction into the Mediterranean Region. *Myriophyllum heterophyllum* is native to the southeast USA and *L. peploides* to South and Central USA and both species are listed as invasive alien species of EU concern (EU 2014). Their native distribution and preferred climate match both risk assessment areas. *Ludwigia peploides* was recently recorded in the Pannonian Region (River Ilova: Buzjak and Sedlar 2018) and has been recognised as posing a severe problem should it expand its current distributional range (Vuković et al. 2021). Additionally, in Croatia, *M. heterophyllum* was recently found in the Mediterranean Region on the Island of Krk (north-eastern Adriatic) in Lake Ponikve (Starmühler 2009; Jasprica et al. 2017), as well as in the River Neretva Delta (Jasprica et al. 2017). It is likely that both species can spread rapidly due to their fast uncontrolled growth and propagation by fragments (Gérard et al. 2014; Gross et al. 2020). Both *L. peploides* and *M. heterophyllum* are tolerant of a wide range of temperatures, substrata, and water quality (Matrat et al. 2004; Hussner and Jahns 2015). The most suitable habitat in Croatia for *L. peploides* is the eastern Pannonian Region, whereas for *M. heterophyllum*, the Mediterranean Region appears to be more suitable (Rodríguez-Merino et al. 2018).

However, under global warming conditions *L. peploides* may accelerate the time of germination of its seeds (Gillard et al. 2017) and for *M. heterophyllum*, the ecologically suitable habitat is likely to increase in both risk assessment areas (Jasprica et al. 2017).

The horizon species *Lemna turionifera*, *Najas guadalupensis*, *Nelumbo nucifera* and *Vallisneria australis* from medium-risk level areas under current climate conditions were predicted to become high-risk under climate change in both risk assessment areas, whereas a high-risk score under such conditions was obtained for horizon *Nymphaea lotus* and extant *Pistia stratiotes* only for the Mediterranean Region. Naturalised populations of *L. turionifera*, *N. nucifera*, *V. australis* and *P. stratiotes* are found in Europe (Mastrantuono and Mancinelli 1999; Hussner 2012; Mesterházy et al. 2021) and can be expected in the risk assessment areas in the near future. Recently, tropical *P. stratiotes* was found in Croatia (Boršić and Rubinić 2018), although its status remains unknown. This species has been found to be established in Slovenia in thermal springs of the Pannonian Region (Jaklič et al. 2020), where it is capable of explosive vegetative spread in early spring. This indicates that *P. stratiotes* can expand its range in similar habitats of the area (Šajna et al. 2007). In addition, global warming may also assist in the process of the species' expansion.

Other screened species including extant *Najas graminea* and horizon *Nymphaea lotus*, *Nymphaea candida*, *Rotala macrandra*, *Rotala rotundifolia* and *Sagittaria subulata* gained the lowest scores in both risk assessment areas. However, considering that some of those species are naturalised in Europe (e.g. *S. subulata*: Hrivnák et al. 2019), their threat of establishment due to the presence of diverse climate types in the risk assessment areas cannot be ruled out. An example is the established population of *N. graminea* on the Island of Cres in the Mediterranean Region (Nikolić 2022), despite this population being localised.

Conclusions

Research on aquatic plants in Croatia is historically fragmented and has not been conducted systematically (Odak and Treer 2000) with the result that, compared to other groups of aquatic organisms, it is almost non-existent (MINGOR 2022; Nikolić 2022). Most of the available reports have been related to aspects of aquatic plants in aquaculture (Bralić 1969; Debeljak et al. 1992), so that there is no research investigating the impacts of non-native aquatic plants on the aquatic ecosystems and biodiversity of Croatia. The removal and reduction of dense mats of aquatic plants, including those of non-native *Elodea canadensis*, in fishponds of the risk assessment areas have been attempted by the use of herbicides and by the introduction of non-native herbivorous fish species for biological control (e.g. grass carp *Ctenopharyngodon idella*) (Đisalov 1961; Bralić 1969), but without real success. Overall, there have been no systematic attempts to eradicate any species of non-native aquatic plants until recently, when a first attempt was made for *Myriophyllum heterophyllum* in the River Neretva Basin in 2021. However, the outcomes of this eradication programme are not yet known.

Despite the recent establishment of monitoring programmes for invasive alien species co-financed by the European Union Cohesion Fund, in the Croatian Catalogue of alien species, data are currently missing for aquatic non-native plants and in relation to

species' description and pathways/vectors of introduction and distribution (MINGOR 2022: https://invazivnevrste.haop.hr/katalog). This suggests that urgent research is necessary in the risk assessment areas of the country to develop management plans for the establishment of rapid control and eradication measures. The present study, therefore, represents the first step towards an increase in the knowledge about the risks poses by the extant and horizon non-native aquatic plants in Croatia, which may allow decision-makers to develop adequate measures for management and control/mitigation.

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Supplementary material 1

Table S1

Authors: Marina Piria, Tena Radočaj, Lorenzo Vilizzi, Mihaela Britvec

Data type: docx file

Explanation note: List of the 55 questions making up the Aquatic Species Invasiveness Screening Kit (AS-ISK).

Link: https://doi.org/10.3897/neobiota.76.83320.suppl1

Supplementary material 2

Combined AS-ISK report for the 24 non-native aquatic plant species screened for their potential risk of invasiveness in the Pannonian and Mediterranean regions of Croatia.

Authors: Marina Piria, Tena Radočaj, Lorenzo Vilizzi, Mihaela Britvec

Data type: pdf file

Explanation note: Combined AS-ISK report for the 24 non-native aquatic plant species screened for their potential risk of invasiveness in the Pannonian and Mediterranean regions of Croatia.

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